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INHIBITION OF MYCOBACTERIUM TUBERCULOSIS ENOYL ACP REDUCTASE InhA BY NOVEL SERIES OF 1,2,4-TRIAZOLE DERIVATIVES

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ABSTRACT

Here we report the *insilico* antitubercular evaluation of a series of novel 1, 2, 4-triazole derivatives. Molecular docking was carried out on enoyl ACP reductase from Mycobacterium tuberculsosis using arguslab, which is one of the key enzymes involved in type II fatty acid biosynthetic pathway of Mycobacterium tuberculosis, an attractive target for designing novel antitubercular agents. Docking study of the compounds were carried out using Argus lab software and then it is loaded in to molegro molecular viewer or pymol viewer to find out hydrogen bonding, hydrophobic, vandervals and actual binding interaction of the molecules on its target InhA. Ligand binding site prediction was carried out using FT-Site server. Compounds 4a6 and 4a7 exhibited the highest antitubercular activity almost close to isoniazid. All other compounds showed good activity.

KEYWORDS: 1,2,4-triazole, InhA, docking.

INTRODUCTION

Bacterial enoyl-acyl carrier protein reductase (ENR) catalyzes an essential step in fatty acid biosynthesis.[1] ENR is an attractive target for narrow-spectrum antibacterial drug discovery because of its essential role in metabolism and its sequence conservation across many bacterial species. In addition, the bacterial ENR sequence and structural organization are distinctly different from those of mammalian fatty acid biosynthesis enzymes. *Mycobacterium tuberculosis* enoyl-ACP reductase (InhA) has been validated as a promising target for antitubercular agents. InhA was identified as an NADH-dependent enoyl-ACP (CoA) reductase specific for long-chain enoyl thioesters and is a member of the Type II fatty acid biosynthesis system, which elongates acyl fatty acid precursors of mycolic acids.[2] Isoniazid (INH), the most prescribed drug to treat tuberculosis (TB), inhibits a NADH-dependent InhA that provides precursors of mycolic acids, which are components of the mycobacterial cell wall. It is a pro-drug that needs activation to form the inhibitory INH-NAD adduct by KatG coding for catalase-peroxidase. The trans-2-enoyl-ACP reductase enzyme of *M. tuberculosis* catalyzes the last step in the elongation cycle of the type II fatty acid synthase (FAS-II) pathway and reduces the 2.3 double bond of trans-2-enoyl-ACP in a NADH-dependent manner. In the present study, special attention was paid to discuss the chemical nature and recent developments of direct InhA inhibitors.[3] The InhA inhibitors reported here have significant inhibitory effects against *Mtb* InhA. Triazole derivatives have been reported to show interesting biological activities such as antibacterial[4,5], antifungal[6], antiviral[7,8], anti convulsant[9], anti-oxidant[10], antitubercular[11], anticancer[12], anti-inflammatory and analgesic[13] activities.

Docking analysis of the crystal structure of InhA performed by using Arguslab software indicates the occupation of substituted triazole derivatives into hydrophobic pocket of InhA enzyme. The analysis of 3D structure allowed us to investigate the effect of different substituent groups at different positions of the common scaffold. Further *in vitro* testing of ligands using biological assays will substantiate the efficacy of ligands that were screened through *in silico* methods.

MATERIALS AND METHODS

All the compounds were constructed using Chem Draw Ultra software, Cambridge Soft Corporation, USA. Version-12.0, 1997-2010. It is a Chem Tech tool used for the drawing of ligand molecules. The crystal structure of INHA receptor used for docking was recovered from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home) (entry code: 4u0j).

Docking study

Lead optimization

Lead optimization was done through insilico Lipinski filter. Molinspiration server was used for this purpose.[14] The structure drawn in the JME editor was subjected to

calculate the druglikeness score through calculate the properties module. The datas are given in the table 1.

Input File Preparations for Energy Minimization of Protein

For each of the protein-ligand complexes chosen for the study, a "clean input file" was generated by removing water molecules, ions, ligands, and subunits not involved in ligand binding from the original structure file. Water molecules were removed because ArgusLab sometimes failed to dock the compounds having water molecules at their binding sites. All hydrogen atoms in the protein were allowed to optimize. The hydrogen locations are not specified by the X-ray structure but these are necessary to improve the hydrogen bond geometries, at the same time maintaining the protein conformation very close to that observed in the crystallographic model. The resulting receptor model was saved to a PDB file. Minimization was performed by geometry convergence function of ArgusLab software performed according to Hartree-Fock calculation method.[15]

Ligand Input File Preparation and Optimization

Ligand input structure was drawn using Chem Draw software. The structure was cleaned in 3D format and energy was minimized. The resulting structure was then saved in "mdl mol" format for molecular docking studies.

Docking Methodology

After the preparation of the protein and ligand, molecular docking studies were performed by ArgusLab 4.0.1 to evaluate the interactions. The active site of protein was obtained from FT Site Server.[16]

ArgusLab 4.0.1

Argus Lab is implemented with shapebased search algorithm. Docking has been done using "Argus Dock" exhaustive search docking function of Argus Lab with. grid resolution of 0.40 ˚ A. Docking precision was set to "Regular precision" and "Flexible" ligand docking mode was employed for each docking run. The stability of each docked pose was evaluated using ArgusLab energy calculations and the number of hydrogen bonds formed.

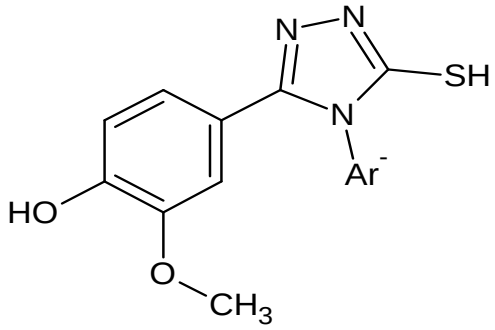
Molecular Docking Study

To perform docking one first needs to define atoms that make up the ligand and the binding sites of the protein where the ligand should bind. The prepared 3D structure of 4u0j protein was downloaded into the ArgusLab program and binding sites were made by choosing "Make binding site for this protein" option. The ligand was then introduced and docking calculation was allowed to run using shape-based search algorithm and AScore scoring function. The scoring function is responsible for evaluating the energy between the ligand and the protein target. Flexible docking was allowed by constructing grids over the binding sites of the protein and energy-based rotation is set for that ligand's group of atoms that do not have rotatable bonds. For each rotation, torsions are created and poses (conformations) are generated during the docking proces. For each complex 10 independent runs were conducted and one pose was returned for each run. The best docking model was selected according to the lowest AScore calculated by ArgusLab and the most suitable binding conformation was selected on the basis of hydrogen bond interactions between the ligand and protein near the substrate binding site. The lowest energy poses indicate the highest binding affinity as high energy produces the unstable conformations.[17]

RESULTS AND DISCUSSION

The least binding energy exhibits the highest activity which has been observed by the ranking of poses generated by AScore scoring function of ArgusLab and is given in Table 2.

Table 1 List of substituents used and Lipinski Rule Analysis



Sr. No	Compound code	R	Log p	H donor (nON)	H acceptor (nOHNH)	Mol. Wt	No of violation
1	4a1	$C_7H_6N_2S$	3.88	6	1	356.43	0
2	4a2	C_6H_7N	2.90	5	1	299.36	0
3	4a3	C_6H_6ClN	3.58	5	1	333.80	0
4	4a4	$C_8H_{11}N$	4.13	5	1	327.41	0
5	4a5	$C_8H_{11}NO$	3.76	6	1	343.41	0

6	4a6	$C_8H_{11}N$	3.94	5	1	327.41	0
7	4a7	$C_{12}H_{11}N$	4.70	5	1	375.45	0
8	4a8	$C_9H_{13}N$	4.53	5	1	341.44	0
9	4a9	C_6H_6ClN	3.74	5	1	333.80	0
10	4a10	C_6H_6BrN	3.71	5	1	378.25	0
11	4a11	$C_6H_6N_2O_2$	3.02	8	1	344.35	0
12	4a12	$C_6H_4F_2N_2O_2$	3.47	8	1	380.33	0
13	4a13	$C_6H_6N_2O_2$	2.86	8	1	344.35	0
14	4a14	C_7H_9N	3.35	5	1	313.38	0
15	4a15	C_7H_9NO	2.96	6	1	329.38	0
16	4a16	$C_6H_5Cl_2N_2$	4.37	5	1	368.25	0
17	4a17	C_7H_8ClN	4.38	5	1	347.83	0
18	4a18	$C_6H_6N_2O$	1.13	8	2	343.37	0
19	4a19	C_6H_6ClN	3.77	5	1	333.80	0
20	4a20	C_6H_6BrN	3.90	5	1	378.25	0

Table 2 Binding energy of designed analogues

Sr. No	Compound code	Binding energy (kcal/mol)	Sr. No	Compound code	Binding energy (kcal/mol)
1	4a1	-10.0158	11	4a11	-8.97826
2	4a2	-10.2326	12	4a12	-9.3862
3	4a3	-10.1786	13	4a13	-8.98143
4	4a4	-10.4643	14	4a14	-9.86762
5	4a5	-11.1221	15	4a15	-8.87301
6	4a6	-12.4773	16	4a16	-11.5795
7	4a7	-11.6543	17	4a17	-10.4037
8	4a8	-10.3722	18	4a18	-9.84153
9	4a9	-8.56208	19	4a19	-11.1951
10	4a10	-8.97826	20	4a20	-10.9328
Std	Isoniazid	-9.548			

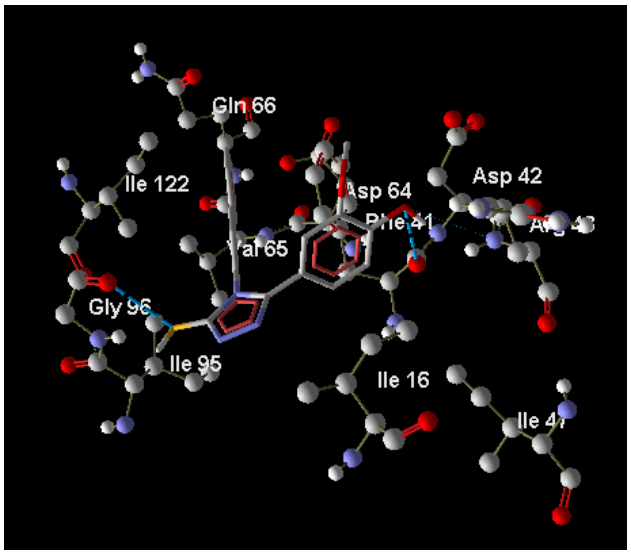


Fig.1 Hydrogen bond interaction of 4a7 with 4u0j

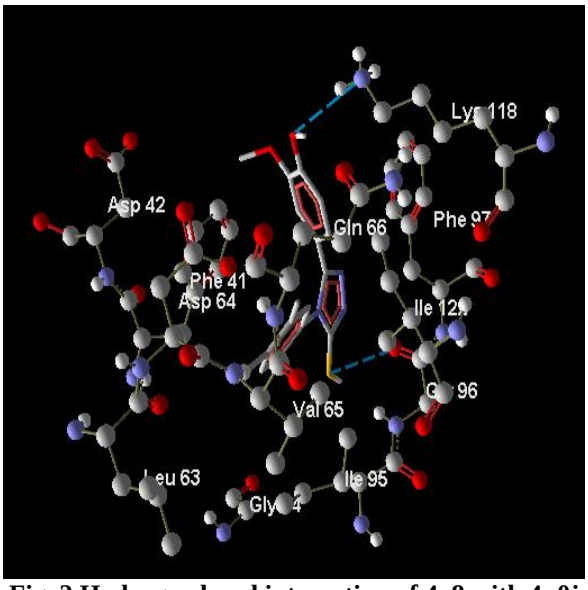


Fig. 2 Hydrogen bond interaction of 4a8 with 4u0j

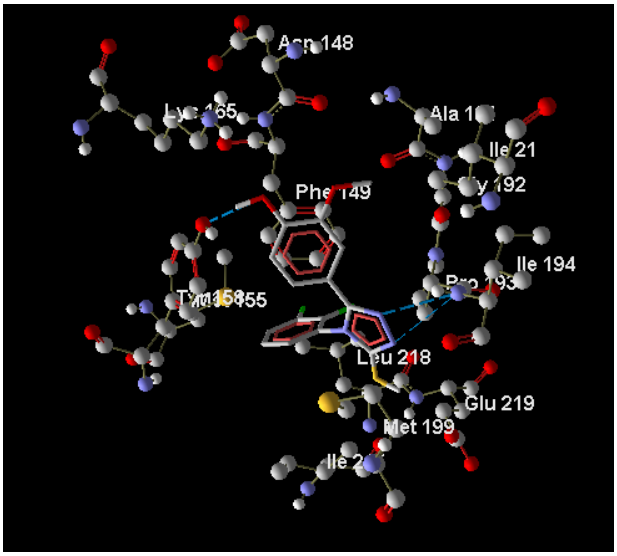


Fig.3 Hydrogen bond interaction of 4a16 with 4u0j

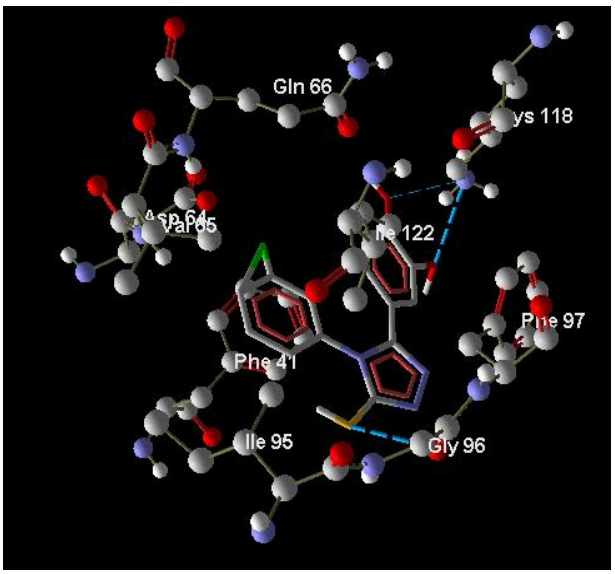


Fig.4 Hydrogen bond interaction of 4a19 with 4u0j

CONCLUSION

Preliminary *in-silico* molecular modeling was carried out with the help of available softwares. All the proposed analogs obeyed Lipinski's Rule of Five. Docking studies were carried out on the proposed analogue to determine the affinity with the enzyme InhA using Argus lab. The analogue 4a6 was found to have higher docking score and significant binding interaction. Molecular docking studies shows that hydrogen bond interaction and hydrophobic interaction plays a crucial role in the biological activity of novel compounds.

REFERENCE

1. Ling LL, Xian J, Ali S, Geng B, Fan J, Mills DM, Arvanites AC, Orgueira H, Ashwell MA, Carmel G, Xiang Y, Moir DT. Identification and characterization of inhibitors of bacterial enoyl-acyl carrier protein reductase. Antimicrob Agents Chemother., 2004 May; 48(5): 1541-1547.
2. Oliveira JS, Vasconcelos IB, Moreira IS, Santos DS, Basso LA. Enoyl reductases as targets for the development of anti-tubercular and anti-malarial agents. Curr Drug Targets, 2007 Mar; 8(3): 399-411.
3. Inturi B, Pujar GV, Purohit MN. Recent Advances and Structural Features of Enoyl-ACP Reductase Inhibitors of Mycobacterium tuberculosis. Arch Pharm (Weinheim). 2016 Nov; 349(11): 817-826.
4. Nareshvarma Seelam, Shrivastava SP, Prasanthi S, Supriya Gupta. Synthesis and *in- vitro* study of some fused 1, 2, 4-triazole derivatives as antimycobacterial agents. Journal of Saudi Chemical Society, 2016; 20: 411–418.
5. Nisha Aggarwal, Rajesh Kumar, Prem Dureja, J.M. Khurana. Synthesis, antimicrobial evaluation and QSAR analysis of novel nalidixic acid based 1,2,4-triazoles derivatives. Eur. J. Med. Chem., 2011; 46(9): 4089-4099.
6. Sheng CQ, Zhang WN, Ji HT, Zhang M, Song YL, Xu H, Zhu J, Miao ZY, Jiang QF, Yao JZ, Zhou YJ, Zhu J, Lv JG. Structure-based optimization of azole antifungal agents by CoMFA, CoMSIA and molecular docking. J Med Chem., 2006; 49: 2512–2525.
7. Wang Z, Wu B, Kuhen KL, Bursulaya B, Nguyen TN, Nguyen DG, He Y. Synthesis and biological evaluations of sulfanyltriazoles as novel HIV-1 non-nucleoside reverse transcriptaseinhibitors. Bioorg Med Chem Lett, 2006; 16: 4174–4177.
8. Martha De La Rosa, Hong Woo Kim, Esmir Gunic, Cheryl Jenket, Uyen Boyle, Yung hyo Koh, Ilia Korboukh, Matthew Allan, Weijian Zhang, Huanming Chen, Wen Xu, Shahul Nilar, Nanhua Yao, Robert Hamatake, Stanley A. Lang, Zhi Hong, Zhijun Zhang, Jean- Luc Girardet, Trisubstituted triazoles as potent non-nucleoside inhibitors of HIV-1 reversetranscriptase. Bioorg Med Chem Lett., 2006; 16: 4444–4449.
9. Dayanand Kadadevar, Chalivaraju KC, Niranjan MS, Chandrasekar Sulthanpur, Santosh Kumar Madinur, Nagaraj. Synthesis of N-(substituted phenyl)-2-[5-phenyl-2H-1, 2, 4-triazol-3ylamino]acetamide as anticonvulsant. Int. J. Chem. Tech. Res., 2011; 3(3): 1064-1069.
10. Rohini Diwedi, Alexander S, Chandrasekar MJN. Rapid and efficient synthesis of microwave assisted synthesis of some bis-1,2,4-triazole derivatives and their antioxidant and anti-inflammatory evaluation. Res. J Pharm. Biol. Chem. Sci, 2011; 2(1): 194-204.
11. Shashikant Pattan, Priyanka Gadhave, Vishal Tambe, Santhosh Dengale, Deepak Thakur. Synthesis and evaluation of some novel 1,2,4-triazole derivatives for antimicrobial, anti-tubercular, anti-inflammatory activities. Ind. J. Chem, 2012; 51B: 297-301.
12. Olcay Bekircan and Nurhan Gumrukcuoglu. Synthesis of some 3,5-diphenyl-4*H*-1,2,4-triazole derivatives as antitumor agents. Ind. J. Chem 2005; 44B: 2107-2113.

13. Goyal PK, Bhandari A, Rana AC, Jain CB. Synthesis, characterization and analgesic activity of some 4*H*-1, 2, 4-triazole derivatives. Int. J. Chem. Tech Res., 2010; 2(4): 1992-1997.
14. Lipinski C.A., F. Lombardo, B.W. Dominy and P. J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev., 2001; 46: 3–26.
15. Joy S, P. S. Nair, R. Hariharan and M. R. Pillai. Detailed comparison of the protein-ligand docking efficiencies of GOLD, a commercial package and arguslab, a licensable freeware. In Silico Biology, 2006; 6(6): 601–605.
16. Kozakov D, Grove LE, Hall DR, Bohnuud T, Mottarella SE, Luo L, Xia B, Beglov D, Vajda S. The FTMap family of web servers for determining and characterizing ligand-binding hot spots of proteins. Nature Protocols. 2015; 10(5): 733-755.
17. Ambreen Hafeez, Zafar Saied Saify, Afshan Naz, Farzana Yasmin and Naheed Akhtar. Molecular Docking Study on the Interaction of Riboflavin (Vitamin B2) and Cyanocobalamin (Vitamin B12) Coenzymes, Journal of Computational Medicine, 2013; Article ID 312183, 5 pages.